

Molecular Data Reveals Rich Diversity of the Sequestrate Genus *Melanogaster* (Boletales, Paxillaceae) with Emphasis on the Species from China

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Research

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Abstract

Melanogaster are ectomycorrhizal fungi characterized by hypogeous fruitbodies. Many ITS rDNA sequences of *Melanogaster* are recovered from molecular surveys of fungal communities, and remain insufficiently identified making it difficult to determine whether these sequences represent conspecific or novel taxa. In this study, the ITS sequences of *Melanogaster* were collected comprehensively and analyzed within ITS-based phylogenetic framework. Twenty-one distinct phylogenetic species can be distinguished based on the ITS phylogeny and a threshold of 98% ITS sequence identity, and most species of *Melanogaster* showed more than 98.1% intraspecific ITS identity and less than 97.9% interspecific identity. Ten species were recognized from China, but combined morphology, nine of which were described and illustrated in this manuscript, including 4 new species (*M. minobovatus* nov. sp., *M. panzhihuaensis* nov. sp., *M. quercus* nov. sp. and *M. tomentellus* nov. sp.), 1 new combination (*M. obvatus* comb. & stat. nov.), and 4 known species (*M. broomeanus*, *M. shanxiensis*, *M. spinisporus*, *M. subglobisporus*). Significantly amendments were added to *M. spinisporus* and *M. subglobisporus*. *M. fusisporus* was added a new mature specimen from type location, and the description and exquisite illustrations were provided also. The diagnostic characteristics of the genus *Melanogaster* and a key to the Chinese species of the genus *Melanogaster* were provided. A 28 Sphylogeny was also conducted to confirm the phylogenetic locations of Chinese *Melanogaster* species and "*Alpova trappei*", consistent with ITS analysis.

Introduction

The genus *Melanogaster* Corda. was erected by Corda (1831), belonging to *Basidiomycota*, *Basidiomycetes*, *Boletales*, *Paxillaceae* (Binder and Hibbett, 2006; Lacheva 2015). The species of this genus have partly or entirely hypogeous fruit bodies, commonly called false truffles, and found on almost all continents (Türkoğlu and Castellano, 2013). Their fruit bodies are tuber-like after dried especially, comprised of a central spore producing region (gleba) consisting of many global chambers surrounded by an outer skin (peridium), and spores are usually dark color. On ripening, their fruit bodies are gelatinous and elastic, the contents of the glebal chambers disintegrate and liquefy to produce a very dark and slimy spore mass, which is quite different with true truffle (Ainsworth 2005).

In China, the first two records are *M. variegates* (Vittad.) Tul. & C. Tul. from Yunnan Province (Keissler and Lohwag 1937) and *M. ambiguus* (Vittad.) Tul. & C. Tul. from Beijing (Teng 1963), but Wang et al. (1995) proposed them as new species *M. ovoidisporus* var. *ovoidisporus* Wang and *M. spinisporus* Wang, respectively. And, at the same time, Wang et al. (1995) described the other five species and varieties from China, including *M. fusisporus* var. *fusisporus* Y. Wang, *M. fusisporus* Wang var. *obovatus* K. Tao, Ming C. Chang & B. Liu, *M. natsii* Wang, K. Tao et Liu, *M. ovoidisporus* Wang var. *angustatosporus* K. Tao, Ming C. Chang & B. Liu, and *M. subglobosporus* K. Tao, Ming C. Chang & B. Liu. In addition, Liu et al. (1989) reported *M. shanxiensis* B. Liu, K. Tao & Ming C. Chang and *M. obovatisporus* B. Liu, K. Tao & Ming C. Chang from Shanxi Province. Yuan and Sun (1996) reported *M. broomeianus* Berk. from Sichuan Province. Up to 1996, a total of 8 species and 2 varieties of the genus *Melanogaster* have been found in China.

With the rapid development of molecular techniques, molecular methods have revolutionized the study of taxonomy and phylogeny of hypogeous fungi (e.g. Bonito et al. 2010; Orihara et al. 2012). However, only a few molecular analyses focused on the taxonomy of gastroid *Paxillineae* (Moreau et al. 2011, 2013). In this study, the internal transcribed spacer region of ribosomal DNA (ITS) and nuclear ribosomal large subunit (nrLSU) were used to identify and analyze the taxonomy and systematics of *Melanogaster*, and our aims are: (1) to clarify the phylogenetic diversity of *Melanogaster* species based on ITS and nrLSU sequences newly obtained from Chinese specimens in this study as well as downloaded from published databases (Genbank, <https://www.ncbi.nlm.nih.gov/genbank/>), and (2) to describe the Chinese species of *Melanogaster*, including 4 new species and 1 new combination.

Materials And Methods

Material studied

Fresh specimens were collected from Shanxi and Yunnan provinces in China. They were dried and deposited in BJTC (Herbarium Biology Department, Capital Normal University). Other specimens were studied from HMAS (Herbarium Mycologicum Academiae Sinicae, Institute of Microbiology, Chinese Academy of Sciences) and KUN (Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences). Macroscopic characters were recorded from fresh and dried specimens. Microscopic characters were examined from dried materials by mounting free-hand sections of basidiomata in 5% KOH, Melzer's reagent (Dring 1971), or 0.1% (w/v) cotton blue in lactic acid. Spore dimensions, excluding ornamentation, were based on 50 spores for each basidioma; measurements are presented as diameter (mean diam \pm SD, n = 50) for each species. For scanning electron microscopy (SEM), ascospores were scraped from the dried gleba, placed onto double-sided tape that was mounted directly on the SEM stub, coated with a platinum-palladium film 8 nm thick using an ion-sputter coater (HITACHI E-1010), and examined with a HITACHI S-4800 SEM.

Molecular methods

Dried basidiomata were crushed by shaking for 45 s at 30 Hz 2–4 times (Mixer Mill MM301, Retsch, Haan, Germany) in a 1.5 ml tube together with one 3 mm diam tungsten carbide ball. Total genomic DNA was extracted using the modified CTAB method (Gardes and Bruns 1993). A total of two nuclear loci were sequenced for this study, including ITS and nrLSU. The ITS region was amplified using the primers for ITS1f/ITS1/ITS4/ITS4B (White et al. 1990; Gardes and Bruns 1993). The primer pair LROR/LR5 (Vilgalys and Hester 1990) was used to amplify the nrLSU fragments. Polymerase chain reactions (PCRs) were performed in 25 μ l reactions containing DNA template 4 μ l, primer (10 μ M/L) 2 μ l each, 2 \times Master Mix (Tiangen Biotech (Beijing) Co. Ltd.) 25 μ l. PCR reactions were implemented as follows: an initial denaturation at 94 $^{\circ}$ C for 3 min; followed by 35 cycles at 94 $^{\circ}$ C for 45 s, 55 $^{\circ}$ C for 45 s, 72 $^{\circ}$ C for 1 min; and a final extension at 72 $^{\circ}$ C for 10 min. The PCR products were purified with a Gel Extraction & PCR Purification Combo Kit (Spin-column) (Biotek, Beijing, China) and then sequenced by Beijing Zhongkexilin Biotechnology Co. Ltd. (Beijing, China) used the same primers as in the PCR application. The products that failed to

be sequenced were cloned into a pLB vector (Tiangen, Beijing) and then sequenced with primers: 23-mer (5' -CGACTCACTATAGGGAGAGCGGC-3') and M13R (5' -AAGAACATCGATTTTCCATGGCAG-3').

Sequence preparation

Total 22 ITS and 14 nrLSU fragments newly generated sequences were assembled and edited using SeqMan (DNA STAR package; DNASTar Inc., Madison, WI, USA) with generic-level identities for sequences confirmed via Blast queries of GenBank. Additional ITS and nrLSU sequences of *Melanogaster* and *Alpova* species were downloaded from GenBank, combined with our sequence database to create ITS and nrLSU phylogeny to illustrate the phylogenetic diversity in *Melanogaster*. The genus search tool in emerencia (<http://www.emerencia.org/>) was used to retrieve insufficiently identified ITS sequences from GenBank, whose pairwise similarity was most similar to identified *Melanogaster* species (Nilsson et al. 2005; Ryberg et al. 2009). The data set of compiled ITS and nrLSU sequences were filtered by removing sequences of poor quality or short length as well as sequences not belonging to *Melanogaster* based on BLAST (Altschul et al. 1997). Details of the taxa, specimens, host, origin and GenBank sequence accessions of ITS and 28S sequences employed in the phylogeny analyses were provided in Table 1 and Table 2 Respectively.

Table 1

Sources of specimens and GenBank accession numbers for sequences used in this study. Sequences newly generated for this study are in bold.

Taxon name	Specimen_voucher	Host	Sporocarp/ECM	Origin	nrDNA-LSU	Study
<i>Alpova alpestris</i>	PAM07082629	<i>Alnus alnobetula</i> subsp. <i>alnobetula</i>	Fruitbody (holotype)	France	NR 132847	Rochet et al. 2011
<i>Alpova alpestris</i>	PAM07082629	<i>Alnus alnobetula</i> subsp. <i>alnobetula</i>	fruitbody	France	HQ714696	Moreau et al. 2011
<i>Alpova alpestris</i>	EDB080829	<i>Alnus alnobetula</i> subsp. <i>alnobetula</i>	fruitbody	France	HQ714775	Moreau et al. 2011
<i>Alpova alpestris</i>	PAM09082201	<i>Alnus alnobetula</i> subsp. <i>alnobetula</i>	fruitbody	France	HQ714777	Moreau et al. 2011
<i>Alpova alpestris</i>	PAM07090501	<i>Alnus alnobetula</i> subsp. <i>suaveolens</i>	fruitbody	France	HQ714691	Moreau et al. 2011
<i>Alpova alpestris</i>	PAM07090501	<i>Alnus alnobetula</i> subsp. <i>suaveolens</i>	fruitbody	France	HQ714692	Moreau et al. 2011
<i>Alpova alpestris</i>	PAM08090302	<i>Alnus alnobetula</i> subsp. <i>suaveolens</i>	fruitbody	France	HQ714721	Moreau et al. 2011
<i>Alpova alpestris</i>	PAM09082802	<i>Alnus alnobetula</i> subsp. <i>alnobetula</i>	fruitbody	France	HQ714776	Moreau et al. 2011
<i>Alpova austroalnicola</i>	LS Dominguez 2290	<i>Alnus acuminata</i>	fruitbody	Argentina	HQ714793	Moreau et al. 2011
<i>Alpova</i> cf. <i>cinnamomeus</i>	PAM09082702	<i>Alnus alnobetula</i> subsp. <i>alnobetula</i>	fruitbody	France	HQ714779	Moreau et al. 2011
<i>Alpova cinnamomeus</i>	Hayward Z5	—	ECM	—	KF836000	Hayward & Horton 2014
<i>Alpova cinnamomeus</i>	BROWN FP73	—	ECM	—	KF835996	Hayward & Horton 2014
<i>Alpova cinnamomeus</i>	MQ18R050-HRL2671- QFB30133	—	fruitbody	Canada	MN992116	Unpublished
<i>Alpova concolor</i>	OSC:65696	—	holotype	—	NR_154686	Hayward&Horton 2014
<i>Alpova concolor</i>	OSC:65696	—	fruitbody	—	KF835994	Hayward & Horton 2014
<i>Alpova concolor</i>	UBC F14673	—	ECM	—	KF835997	Hayward & Horton 2014
<i>Alpova corsicus</i>	PAM07090803	<i>Alnus alnobetula</i> subsp. <i>suaveolens</i>	fruitbody	France	HQ714699	Moreau et al. 2011
<i>Alpova corsicus</i>	FR2	<i>Alnus cordata</i>	fruitbody	France	HQ714766	Moreau et al. 2011
<i>Alpova corsicus</i>	PAM05102601	<i>Alnus cordata</i>	fruitbody	France	HQ714698	Moreau et al. 2011
<i>Alpova diplophloeus</i>	Hayward1	—	ECM	—	KF835992	Hayward & Horton. 2014
<i>Alpova diplophloeus</i>	Hayward 2	—	ECM	—	KF836001	Hayward & Horton 2014
<i>Alpova diplophloeus</i>	OSC59767:Trappe11088	—	fruitbody	—	DQ989497	Unpublished
<i>Alpova komoviana</i>	LIP:PAM10081201	—	fruitbody	—	JQ436850	Moreau et al. 2013
<i>Alpova</i> sp.	OSC:59767	—	fruitbody	—	KF835995	Hayward & Horton 2014
<i>Alpova</i> sp.	—	—	ECM	Spain	AJ419181	Martin & Raidl 2002
<i>Alpova</i> sp.	TK1622	—	fruitbody	USA	AY558739(its1)	Unpublished
<i>Alpova trappei</i>	OSC JMT 16394	—	fruitbody	USA	AF074920	Grubishaet al. 2002
<i>Alpova trappei</i>	SNF105	—	fruitbody	USA	AY558738(its1)	Unpublished
<i>Alpova trappei</i>	OSC158328 JLF2124	—	fruitbody	USA	MN984306	Unpublished

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<i>Alpova trappei</i>	OSC158329 JLF4410	–	fruitbody	USA	MN984307	Unpublished
<i>Gyrodon lividus</i>	–	–	–	Germany	DQ534568	Binder & Hibbett 2006
<i>M. ambiguus</i>	B-2220	–	fruitbody	Hungary	AJ555510	Unpublished
<i>M. ambiguus</i>	51745	–	fruitbody	Hungary	AJ555511	Unpublished
<i>M. ambiguus</i>	B-1599	–	fruitbody	Hungary	AJ555512	Unpublished
<i>M. ambiguus</i>	B-2409	–	fruitbody	Hungary	AJ555514	Unpublished
<i>M. ambiguus</i>	Ch12	<i>Corylus avellana</i>	ECM	Poland	KX438335	Unpublished
<i>M. broomeanus</i>	BJTC FAN909	<i>Lespedeza bicolor</i>	fruitbody	China	✓	In this study
<i>M. broomeanus</i>	BJTC FAN1230-A	<i>Pinus bungeana</i>	fruitbody	China	✓	In this study
<i>M. broomeanus</i>	BJTC FAN1230-B	<i>Pinus bungeana</i>	fruitbody	China	✓	In this study
<i>M. broomeianus</i>	48353	–	fruitbody	Hungary	AJ555516	Unpublished
<i>M. broomeianus</i>	B-1616	–	fruitbody	Hungary	AJ555517	Unpublished
<i>M. broomeianus</i>	RBG Kew K(M)124251	–	fruitbody	UK	EU784370	Brock et al. 2009
<i>M. broomeianus</i>	B-2331	–	fruitbody	Hungary	AJ555530	Unpublished
<i>M. broomeianus</i>	23633	–	fruitbody	Hungary	AJ555518	Unpublished
<i>M. cf. intermedius</i>	RBG Kew K(M)122480	–	fruitbody	UK	EU784371	Brock et al. 2009
<i>M. eurypermus</i>	–	–	fruitbody	USA:	AY918954	Frank et al. 2006
<i>M. intermedius</i>	B-1770	–	fruitbody	Hungary	AJ555515	Unpublished
<i>M. intermedius</i>	RBG Kew K(M)130202	–	fruitbody	UK	EU784372	Brock et al. 2009
<i>M. intermedius</i>	MT48	<i>Fagus sylvatica</i>	ECM	Germany	KX168661	Unpublished
<i>M. luteus</i>	PAM09082801	<i>Alnus incana</i> subsp. <i>incana</i>	fruitbody	France	HQ714780	Moreau et al. 2011
<i>M. luteus</i>	Mon06	<i>Alnus incana</i> subsp. <i>incana</i>	fruitbody	Montene	HQ714794	Moreau et al. 2011
<i>M. macrosporus</i>	cl-94	–	fruitbody	Hungary	AJ555526	Unpublished
<i>M. macrosporus</i>	B-2254	–	fruitbody	Hungary	AJ555528	Unpublished
<i>M. minobovatus</i>	BJTC FAN911	<i>Pinus bungeana</i>	fruitbody(holotype)	China	Not submitted	In this study
<i>M. obovatus</i>	BJTC FAN1102	<i>Quercus variabilis</i>	fruitbody	China	Not submitted	In this study
<i>M. obovatus</i>	BJTC FAN1091	<i>Quercus variabilis</i>	fruitbody(epitype)	China	Not submitted	In this study
<i>M. obovatus</i>	BJTC FAN1208	<i>Quercus</i> sp.	fruitbody	China	Not submitted	In this study
<i>M. panzhihuaensis</i>	HMAS81915	<i>Pinus yunnanensis</i>	fruitbody(holotype)	China	Not submitted	In this study
<i>M. quercus</i>	BJTC FAN808	<i>Quercus</i> sp.	fruitbody	China	Not submitted	In this study
<i>M. quercus</i>	BJTC FAN809	<i>Quercus</i> sp.	fruitbody(holotype)	China	Not submitted	In this study
<i>M. rivularis</i>	PAM08090514	<i>Alnus glutinosa</i>	fruitbody	France	HQ714731	Moreau et al. 2011
<i>M. rivularis</i>	PAM08090514	<i>Alnus glutinosa</i>	fruitbody	France	HQ714767	Moreau et al. 2011
<i>M. rivularis</i>	PAM08090514	<i>Alnus glutinosa</i>	fruitbody	France	NR_132848	Moreau et al. 2011
<i>M. shanxiensis</i>	HMAS81910	<i>Lespedeza bicolor</i>	fruitbody(holotype)	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN1085	<i>Quercus variabilis</i>	fruitbody	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN1092	<i>Quercus variabilis</i>	fruitbody	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN936-A	<i>Pinus bungeana</i>	fruitbody	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN936-B	<i>Pinus bungeana</i>	fruitbody	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN936-C	<i>Pinus bungeana</i>	fruitbody	China	Not submitted	In this study

Taxon name	Specimen_voucher	Host	Sporocarp/ECM	Origin	nrDNA-LSU	Study
<i>M. spinisporus</i>	BJTC FAN938	<i>Pinus bungeana</i>	fruitbody	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN941-A	<i>Ulmus</i> sp.	fruitbody(epitype)	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN941-B	<i>Ulmus</i> sp.	fruitbody	China	Not submitted	In this study
<i>M. subglobisporus</i>	HMAS81919	—	fruitbody(epitype)	China	Not submitted	In this study
<i>M. tomentellus</i>	BJTC FAN539	<i>Pinus</i> sp.	fruitbody(holotype)	China	Not submitted	In this study
<i>M. tuberiformis</i>	B-1295	—	fruitbody	Romania	AJ555527	Unpublished
<i>M. variegatus</i>	B-1438	—	fruitbody	Hungary	AJ555523	Unpublished
<i>M. variegatus</i>	B-1348	—	fruitbody	Hungary	AJ555534	Unpublished
<i>M. vittadinii</i>	33090	—	fruitbody	Hungary	AJ555525	Unpublished
<i>M. ambiguus</i>	HMAS68237	—	fruitbody	UK	Not submitted	In this study
<i>Melanogaster</i> sp.	SOC1324	<i>Quercus garryana</i>	—	USA:	JN022499	Unpublished
<i>Melanogaster</i> sp.	LM3494	<i>Quercus</i> sp.	ECM	France	KM576463	Suz et al. 2014
<i>Melanogaster</i> sp.	OSC 59776	—	fruitbody	USA	KT968555	Unpublished
<i>Melanogaster</i> sp.	OSC 59778	—	fruitbody	USA	KT968556	Unpublished
<i>Melanogaster</i> sp.	Melanog002FRA	<i>Alnus cordata</i>	ECM	France	KU924526	Unpublished
<i>Melanogaster</i> sp.	Melanog004FRA	<i>Alnus cordata</i>	ECM	France	KU924527	Unpublished
<i>Melanogaster</i> sp.	Melanog005FRA	<i>Alnus cordata</i>	ECM	France	KU924528	Unpublished
<i>Melanogaster</i> sp.	Melanog006FRA	<i>Alnus cordata</i>	ECM	France	KU924529	Unpublished
<i>Melanogaster</i> sp.	Melanog007FRA	<i>Alnus cordata</i>	ECM	France	KU924530	Unpublished
<i>Melanogaster</i> sp.	Melanog008FRA	<i>Alnus cordata</i>	ECM	France	KU924531	Unpublished
<i>Melanogaster</i> sp.	Melanog011FRA	<i>Alnus cordata</i>	ECM	France	KU924533	Unpublished
<i>Melanogaster</i> sp.	Melanog012FRA	<i>Alnus cordata</i>	ECM	France	KU924534	Unpublished
<i>Melanogaster</i> sp.	Melanog018FRA	<i>Alnus alnobetula</i>	ECM	France	KU924535	Unpublished
<i>Melanogaster</i> sp.	Melanog019FRA	<i>Alnus alnobetula</i>	ECM	France	KU924536	Unpublished
<i>Melanogaster</i> sp.	MT15	<i>Fagus sylvatica</i>	ECM	Germany:	KX168646	Unpublished
<i>Melanogaster</i> sp.	LMKR1187	<i>Pinus sylvestris</i>	ECM	UK	MF352733	Suz et al. 2017
<i>Melanogaster</i> sp.	SOC1321	<i>Quercus garryana</i>	fruitbody	USA:	JN022515	Unpublished
<i>Nealpova rubescens</i>	16942	—	fruitbody	Italy	JF908774	Osmundson et al. 2013
<i>Nealpova rubescens</i>	M. Zotti 2oct2008	<i>Fagus sylvatica</i>	fruitbody	—	JQ436851	Moreau et al. 2013
<i>Paragyrodon sphaerosporus</i>	MB06-066	—	—	USA	GU187540	Binder et al. 2010
<i>Paxillus adelphus</i>	TL:127-0003	<i>Alnus glutinosa</i>	fruitbody	France	NR_158830	Jargeat et al. 2016
<i>Paxillus rubicundulus</i>	K:M190474	<i>Alnus</i>	fruitbody	UK	NR_147640	Jargeat et al. 2016
uncultured <i>Alpova</i>	—	<i>Alnus alnobetula</i> subsp. <i>crispa</i>	ECM	Canada	MK285738	Unpublished
uncultured <i>Alpova</i>	—	<i>Alnus rhombifolia</i> or <i>Betula occidentalis</i>	ECM	USA	JX198512	Bogar et al. 2013
Uncultured <i>Boletaceae</i>	clone B8	<i>Lithocarpus densiflorus</i>	ECM	USA	DQ273364	Bergemann et al. 2006
Uncultured <i>Boletaceae</i>	LP110023	<i>Quercus liaotungensis</i>	ECM	CHINA	JQ318611	Unpublished
Uncultured <i>Boletaceae</i>	—	<i>Lithocarpus densiflorus</i>	ECM	USA:	DQ273366	Bergemann et al. 2006

Taxon name	Specimen_voucher	Host	Sporocarp/ECM	Origin	nrDNA-LSU	Study
Uncultured ectomycorrhiza (<i>Melanogastraceae</i>)	E3	—	soil	Denmark	AM159585	Kjoller et al. 2006
Uncultured ectomycorrhizal fungus	L7139a	alder species	ECM	Europe	JN197963	Unpublished
Uncultured ectomycorrhizal fungus	S376	alder species	ECM	—	JN198097	Unpublished
Uncultured ectomycorrhizal fungus	S414	alder species	ECM	Europe	JN198124	Unpublished
Uncultured ectomycorrhizal fungus	ecm_Jo9V422	<i>Alnus</i> sp.	ECM	France	JX989839	Roy et al. 2013
Uncultured ectomycorrhizal fungus	ecm_HL9I116	<i>Alnus</i> sp.	ECM	France	JX989860	Roy et al. 2013
Uncultured ectomycorrhizal fungus	ecm_HL9I510	<i>Alnus</i> sp.	ECM	France	JX989867	Roy et al. 2013
Uncultured ectomycorrhizal fungus	ecm_AZA815	<i>Alnus</i> sp.	ECM	France	JX989932	Roy et al. 2013
Uncultured ectomycorrhizal fungus	ecm_FS511	<i>Alnus</i> sp.	ECM	France	JX990028	Roy et al. 2013
Uncultured ectomycorrhizal fungus	ecm_Co8J3C306	<i>Alnus</i> sp.	ECM	France	JX990041	Roy et al. 2013
Uncultured ectomycorrhizal fungus	ecm_AR9I525	<i>Alnus</i> sp.	ECM	France	JX990064	Roy et al. 2013
Uncultured fungus	TKSCAT1649	<i>Glaucomys sabrinus</i>	environmental_sample: small mammal scat	USA	AY558728 (its1)	Unpublished
Uncultured <i>Melanogaster</i>	MFT57	<i>Fagus sylvatica</i>	ECM	Germany	FJ403505	Pena et al. 2010
Uncultured <i>Melanogaster</i>	—	—	environmental_sample	Iran	FR852281	Bahram et al. 2012
Uncultured <i>Melanogaster</i>	QL045	<i>Quercus liaotungensis</i>	ECM	China	HM105535	Wang et al. 2012
Uncultured <i>Melanogaster</i>	clone S9	—	ECM	Germany	HQ336669	Unpublished
Uncultured <i>Melanogaster</i>	clone vial332	<i>Pseudotsuga menziesii</i>	ECM	Canada	JN652972	Unpublished
Uncultured <i>Melanogaster</i>	ECM_alnus_Melalute	<i>Alnus</i> sp.	ECM	France	JQ890286	Roy et al. 2013
Uncultured <i>Melanogaster</i>	ECM_alnus_Melarivu	<i>Alnus</i> sp.	ECM	France	JQ890287	Roy et al. 2013
Uncultured <i>Melanogaster</i>	Mv	—	forest soil	Germany	JX030011	Unpublished
Uncultured <i>Melanogaster</i>	MTH36	<i>Fagus sylvatica</i>	ECM	Germany	KT020803	Unpublished
Uncultured <i>Melanogaster</i>	clone UVIC42	<i>Pseudotsuga menziesii</i>	environmental_sample	Canada	KT272124	Kranabetter et al. 2015

Table 2

Sources of specimens and GenBank accession numbers for sequences used in this study. Sequences newly generated for this study are in bold.

Taxon name	Specimen_voucher	Host	Sporocarp/ECM	Origin	nrDNA-LSU	Study
<i>Alpova australis</i>	CORD LSD 2291	–	–	–	AY377575	Nouhra et al. 2005
<i>Alpova australis</i>	CORD LSD 2290	–	–	–	AY377574	Nouhra et al. 2006
<i>Alpova diplophloeus</i>	OSC 34455	–	fruitbody	–	AY377573	Nouhra et al. 2007
<i>Alpova diplophloeus</i>	–	–	–	–	AF352035	Jarosch & Besl 2001
<i>Alpova diplophloeus</i>	OSC JMT 17685	–	fruitbody	–	AF071454	Grubisha et al. 2001
<i>Alpova diplophloeus</i>	OSC 93524	–	fruitbody	–	AY377571	Nouhra et al. 2005
<i>Alpova diplophloeus</i>	OSC 39950	–	fruitbody	–	AY377572	Nouhra et al. 2006
<i>Alpova trappei</i>	OSC JMT 16394	–	fruitbody	–	AF071456	Grubisha et al. 2001
<i>Alpova trappei</i>	28042	–	–	–	KF030307	Nuhn et al. 2013
<i>M. broomeanus</i>	BJTC FAN1230-A	<i>Pinus bungeana</i>	fruitbody	China	Not submitted	In this study
<i>M. minobovatus</i>	BJTC FAN911	<i>Pinus bungeana</i>	fruitbody(holotype)	China	Not submitted	In this study
<i>M. obovatus</i>	BJTC FAN1091	in the soil under <i>Quercus variabilis</i> and <i>Pinus tabuliformis</i>	fruitbody(epitype)	China	Not submitted	In this study
<i>M. obovatus</i>	BJTC FAN1208	in the soil under <i>Quercus variabilis</i> and <i>Pinus tabuliformis</i>	fruitbody	China	Not submitted	In this study
<i>M. obovatus</i>	BJTC FAN1102-A	<i>Quercus variabilis</i>	fruitbody	China	Not submitted	In this study
<i>M. obovatus</i>	BJTC FAN1102-B	<i>Quercus variabilis</i>	fruitbody	China	Not submitted	In this study
<i>M. panzhihuaensis</i>	HMAS81915	<i>Pinus yunnanensis</i>	fruitbody(holotype)	China	Not submitted	In this study
<i>M. quercus</i>	BJTC FAN808	<i>Quercus</i> sp.	fruitbody	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN941-A	<i>Ulmus</i> sp.	fruitbody(epitype)	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN941-B	<i>Ulmus</i> sp.	fruitbody	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN936-A	<i>Pinus bungeana</i>	fruitbody	China	Not submitted	In this study
<i>M. spinisporus</i>	BJTC FAN936-B	<i>Pinus bungeana</i>	fruitbody	China	Not submitted	In this study
<i>M. tomentellus</i>	BJTC FAN539	<i>Pinus</i> sp.	fruitbody(epitype)	China	Not submitted	In this study
<i>M. ambiguus</i>					AF352046	Jarosch & Besl. 2001
<i>M. broomeianus</i>					AF098383	Bresinsky et al. 1999
<i>Melanogaster</i> sp.	OSC 59776			USA	KT968611	Unpublished
<i>Melanogaster</i> sp.	OSC 59778			USA	KU321864	Unpublished
<i>M. tuberiformis</i>	OSC JMT 9666				AF074919	Grubisha et al. 2001

Taxon name	Specimen_voucher	Host	Sporocarp/ECM	Origin	nrDNA-LSU	Study
<i>M. tuberiformis</i>	strain 628				AF167679	Jarosch & Bresinsky 1999
<i>M. variegatus</i>				Germany	DQ534668	Binder & Hibbett 2006
<i>Paxillus involutus</i>	RV98.135				AY612815	Unpublished
Uncultured <i>Alpova</i>	clone 1_436	<i>Alnus acuminata</i> and <i>Alnus jorullensis</i> forest (<i>Pinus</i> present)	environmental_sample	Mexico	HQ271392	Unpublished
Uncultured <i>Melanogaster</i>	04M7K057		environmental_sample_soil	Germany	FR750834	Kruger et al. 2012
Uncultured <i>Melanogaster</i>	H04M7K57		environmental_sample_soil	Germany	HF676136	Unpublished
Uncultured <i>Melanogaster</i>	H12M7K29		environmental_sample soil	Germany	HF677077	Unpublished

Phylogenetic analysis

Two data sets, ITS and nrLSU, were compiled to identify *Melanogaster* species and investigate relationships among species. The taxa *Gyrodon lividus*, *Paxillus rubicundulus*, *P. adelphus* and *Paragyrodon sphaerosporus* were selected as outgroups for ITS Phylogenetic analysis and *Paxillus involutus* for nrLSU. The ITS and nrLSU sequences were aligned using the MAFFT v.7.110 online program (<https://www.ebi.ac.uk/Tools/msa/mafft/>) and manually adjusted for maximum similarity in Se-AL version.2.03a. (Rambaut 2000). The datasets were manually edited at misaligned sites. Poorly aligned sites were identified by Gblocks v.0.91b (Castresana 2000; using default options except BAllowed Gap Positions = half) with default parameters. All identified ambiguous sites were excluded before the phylogenetic analysis. We conducted maximum likelihood (ML) and Bayesian inference (BI) analyses on the two data sets. The alignments and phylogenetic trees were deposited in TreeBASE (??).

Maximum likelihood (ML) analysis on the dataset was done using RAxML 8.0.14 (Stamatakis et al. 2005; Stamatakis 2006, 2014) and the GTR + G + I substitution model with parameters unlinked. The ML bootstrap replicates (1000) were computed in RAxML using a rapid bootstrap analysis and search for the best-scoring ML tree. The ML trees were viewed with TreeView32 (Page 2001). Clades with bootstrap support (BS) $\geq 70\%$ were considered significant and highly supported (Hillis and Bull 1993).

Bayesian inference (BI) was performed with MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) as an additional method of determining branch support.

BI analyses were performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) based on the best substitution models determined by MrModeltest 2.3 (Nylander 2004), which were GTR + I + G for the ITS data set and SYM + I + G for the nrLSU data set. Two independent runs of four chains were conducted for 1 400 000 (for ITS) and for 580 000 (for nrLSU) Metropolis-coupled Markov chain Monte Carlo (MCMCMC) generations with the default setting. The average standard deviations of split frequency (ASDSF) values were far lower than 0.01 at the end of the generations. Trees were sampled every 100 generations after burn-in (well after convergence), and 50% majority-rule consensus trees were constructed and visualized with TreeView (32 bit) (Page 2001). Clades with Bayesian posterior probability (PP) $\geq 95\%$ were considered significant (Alfaro et al. 2003).

Results

Phylogenetic analysis

The ITS dataset contained 128 sequences (22 generated in this study) and comprised 604 sites, of which 284 were variable sites, and 237 of these variable sites were informative. ML and BI analyses produced identical tree topologies, and only the tree derived from the ML analyses is shown (Fig. 1).

The sequences of *Melanogaster* were grouped into a clade together with 4 sequences labeled as "*Alpova trappei*" with support values of BS = 77 and PP = 1.00, then further clustered with the other sequences of *Alpova* with a strong support value (BS = 100). The sequences of *Alpova* employed in this analysis, except the samples of "*Alpova trappei*", formed two independent clades with strong support. In *Melanogaster* clade, a total 21 phylotypes (marked A–U) can be recognized based on a species delimitation of 98% ITS similarity and strong supported clades of the ITS-based ML tree (Fig. 1). Nine of the 21 phylotypes matched known species, while 12 phylotypes represented uncertain or undescribed species, of which 5 species were described and illustrated as new taxa from China in this study. Phylotype A was designated as *M. intermedius* as it contained 2 reliably identified sequences EU784372 and EU784371 of *M. intermedius* published by Brock et al. (2009), and had a strong support (BS = 97/PP = 1.00); Phylotype B contained 2 ectomycorrhizal (ECM) sequences of USA and Canada, one environmental sequence of Canada and 3 sequences of inaccurately identified specimens from USA, showed more than 98.18% ITS similarity among them and less than 97.23% ITS similarity with other phylotypes. It also had a strong support (BS = 99/PP = 1.00) and could represent a new taxon from North America, marked *M. sp.1* here (Fig. 1); Phylotype C contained 4 sequences, 2 from ECM and environmental sample respectively, one labeled as *Melanogaster ambiguus* from an unpublished study and one sequence was newly obtained in the present work from a specimen of *M. ambiguus* (collected

by Richard in Wiltshire, UK, 1987). These sequences showed more than 99.26% ITS similarity and less than 97.91% ITS similarity with other phylotypes, with a moderate support value (BS = 75). Combined the morphologic features of the specimen of *M. ambiguus* we therefore recognized phylotype D as *M. ambiguus*. Phylotype D contained 4 sequences labeled as *Melanogaster ambiguus* and *M. broomeanus* from an unpublished study about Hungary specimens. These sequences showed more than 99.02% ITS similarity and less than 97.9% ITS similarity with other phylotypes, with a strong support (BS = 94/PP = 1.00). It could represent a new taxon from Hungary, marked *M. sp.2* here; Phylotype E contained one ECM sequences from Poland and 2 sequences of inaccurately identified specimens of Hungary. The 3 sequences showed more than 99.29% ITS similarity among them and less than 97.74% with other phylotypes. It could represent a new taxon from Central Europe, marked *M. sp.3* here; Phylotype F was designated as *M. eurypermus*. For it contained one sequence of *M. eurypermus* published by Frank et al. (2006), and the sequence showed less than 94.53% ITS sequence similarity to other *Melanogaster* species; Phylotype G and N contained one sequence respectively, a sequence (JN022515) of inaccurately identified specimen of USA, and an unpublished ECM sequence (JN198097). The 2 sequences showed 94.53% ITS similarity to each other even with other species of *Melanogaster*. The 2 phylotypes could be new taxa of *Melanogaster*, marked *M. sp.4* and *M. sp.5* respectively; Phylotype H contained 8 sequences obtained from several new collections of Shanxi Province, China, which were identified as *M. spinisporus* morphologically in this study. The sequences shared more than 98.78% ITS similarity, and less than 92.03% ITS similarity to other phylotypes, convincing us that it was a distinct species. Therefore, we recognized phylotype H as *M. spinisporus*; Phylotype I contained 3 sequences obtained from 3 specimens newly collected from Shanxi Province, which was different from all of known species of *Melanogaster* morphologically. The sequences showed more than 99.35% ITS similarity among them and less than 97.9% ITS similarity to other phylotypes. For this, the new taxon *M. obovatus* was proposed in the present study; Phylotype J contained 2 sequences obtained from 2 specimens newly collected from Shanxi Province, China, which could be distinguished from other species of *Melanogaster* morphologically. The sequences showed more than 99.75% ITS similarity among them and less than 97.9% ITS similarity to other phylotypes. Therefore, the new taxon *M. quercus* was proposed for it; Phylotypes K and M contained one sequences newly extracted from holotypes of Chinese species *M. shanxiensis* and *M. subglobisporus* respectively. The two sequences showed less than 97.91% and 92.24% ITS similarity to other phylotypes respectively, clarifying their phylogenetic positions. Therefore, phylotypes K and M were identified *M. shanxiensis* and *M. subglobisporus* respectively; Phylotype L contained one sequence extracted from the specimen (BJTC FAN539) newly collected in Yunnan province, China, which was distinguished from other species of *Melanogaster* obviously in morphology. The sequence showed less than 95.33% ITS sequence similarity to other phylotypes, indicating it is a distinct species in *Melanogaster*. And the new species *M. tomentellus* was thus proposed for it; Phylotype O contained 15 sequences, including the holotype sequence (NR_132848) of *M. rivularis*, showed more than 99.02% ITS similarity among each other and less than 96.76% ITS similarity to other species of genus *Melanogaster*. Therefore, phylotype O was designated as *M. rivularis* with no doubt; Phylotype P contained 11 sequences, including two sequences (HQ714780, HQ714794) from accurately identified specimens of *M. luteus* published by Moreau et al. (2011), showed more than 99.41% ITS similarity among each other and less than 97.07% ITS similarity to other species of genus *Melanogaster*. For the above reasons, phylotype P was recognized as *M. luteus*; Phylotype Q was designated as *M. broomeanus*, containing 11 sequences of which 3 sequences were newly obtained from the specimens of China in this work and a reliably identified sequence of *M. broomeanus* (EU784370) published by Brock et al. (2009). The 11 sequences showed more than 98.09% ITS similarity and less than 95.73% ITS similarity to other species of *Melanogaster*. Consequently, we recognized phylotype Q as *M. broomeanus* based on morphology and phylogenetic analysis; Phylotype R contained only a sequence from ectomycorrhizae (ECM) of *Quercus liaotungensis* Koidz in China, shared less than 94.79% ITS similarity to other species of *Melanogaster*. It should represent a potential new taxon from China, marked *M. sp.6* here; Phylotype S contained 7 sequences of USA, one sequence from ECM of *Lithocarpus densiflorus* and 4 sequences extracted from the specimens labeled as "*Alpova trappei*". Evidently, these sequences of "*Alpova trappei*" did not cluster with *Alpova* sequences in this analysis, implying they represented the *Melanogaster* species rather than *Alpova* species. Thus Phylotype S could represent a potential new taxon of *Melanogaster* from USA, marked *M. sp.7* here; Phylotype T contained 2 sequences, one extracted from the specimen (BJTC FAN911) newly collected in Shanxi province, China and another sequences (JQ318611) obtained from ectomycorrhizae of *Quercus liaotungensis* in Loess Plateau, China. They showed 99% ITS sequence similarity and less than 91.72% ITS similarity to other species of *Melanogaster*, for which a new species *M. minobovatus* was proposed; Phylotype U was represented by one sequence newly obtained from the specimen of Sichuan province, China, which was significantly different from other species of *Melanogaster* morphologically. In addition, the sequence showed less than 89.89% to other species of genus *Melanogaster*, indicating it was a distinct species. Thus, *M. panzhihuaensis* was proposed for it.

Phylogeny based nrLSU sequences were analyzed because there were the samples of *Alpova trappei* grouped into *Melanogaster* clade rather than *Alpova* clade in ITS-based phylogeny although there were a small number of available sequences. The nrLSU dataset contained 35 sequences (14 generated in this study) and comprise 772 sites, of which 103 variable sites, and 64 of these variable sites were informative. ML and BI analyses produced identical tree topologies, and only the tree derived from the ML analyses is shown (Fig. 2). Two clades were recognized in this tree, respectively representing *Melanogaster* and *Alpova*, and the sequences labeled as "*Alpova trappei*" (marked with pentagram (⬠) symbol) were placed in *Melanogaster* clade rather than *Alpova* clade, consistent with that of the ITS-based phylogenetic analysis (Fig. 1). The other sequences of *Alpova* cluster an independent clade with a strong support clade (BS = 97, PP = 1.00). These further confirmed that the samples of *Alpova trappei* represented the species of the genus *Melanogaster*. Moreover, the Chinese *Melanogaster* sequences were grouped in *Melanogaster* clade as those observed on the ITS tree (Fig. 1).

Taxonomy

Melanogaster broomeanus Berk., in Montagne, *Annls Sci. Nat., Bot.*, sér. 2 20: 377 (1843) Fig. 3, a–f

≡ *Melanogaster ovoidisporus* var. *angustatisporus* K. Tao, Ming C. Chang & B. Liu, *Journal of Shanxi University, Natural Science* 18(4): 449 (1995). *syn. nov.*
 Type:—CHINA. SHANXI PROV.: Jincheng City, Yangcheng County, Mang River National Nature Reserve, in soil under *Quercus* sp., 29 May 1988, *M.-c. Chang* 222 (HMAS83258 ex MHSU2022).

Description

Basidiomata 1.5–2.5 × 2–2.5 cm, hypogeous, subglobose to irregularly globose, yellow-brown, becoming blackish where handled, without distinct basal zone; rhizomorphs few, brown; odor no record. Gleba yellow brown when fresh, black when dry, with sterile white veins scattered throughout. Peridium single-layered, 150–400 µm thick, composed of hyphae, hyphae generally parallel to the surface of peridium but loosely interwoven, pale yellow to whitish, 5–12.5 µm in diam, clamp connections abundant; Trama plates of hyaline interwoven hyphae, 2.5–5 µm in diam, thin-walled, tending to be gelatinized. Basidia 16.5–22.2 × 5.0–6.7 µm, light yellow clavate-capitate when young, 4-spored, sterigmata short, collapsed and disappeared at maturity. Basidiospores ellipsoid or oblong to cylindrical, 4.4–7.8 × 3.3–4.7 µm (avX = 6.0 ± 1.0 × 3.9 ± 0.36, n = 50), Q = 1.3–1.9 (avQ = 1.5 ± 0.2, n = 50), deep brown at maturity, walls 0.5 µm thick, smooth, apex obtuse, the base broadly truncate-cupped, with very short remnants of sterigmata.

Habit, habitat and distribution

July to September, in soil under *Quercus liaotungensis*, *Pinus bungeana* Zucc. ex Endl., *Lonicera japonica* Thunb. Warm temperate zone and cold temperate zone of North China.

Notes: *Melanogaster broomeanus* is diagnosed by its yellow-brown basidiomata, single-layered peridium composed of interwoven hyphae, light brown ellipsoid or oblong to cylindrical basidiospores. The specimens (BJTC FAN909, BJTC FAN1230), newly collected from Shanxi Province, China, morphologically fit well with the characteristics of *M. broomeanus*, and phylogenetically their ITS sequences were clustered in the *M. broomeanus* clade with strong support (BS = 99/PP = 1.00) (Fig. 1), suggesting the two Chinese collections represented the Europe species *M. broomeanus*. *M. ovoidisporus* var. *angustatisporus* is described by Wang et al. (1995) based on the specimens from Chinese Shanxi Province, but their morphological traits described by authors is very similar to *M. broomeanus*. In this study, we re-examined the morphology of holotype and paratypes cited by Wang et al. (1995), and compared them with our new collections (BJTC FAN909, BJTC FAN1230) from Shanxi Province, and as a result, there are not any obvious differences to be revealed among these specimens in morphology. Unfortunately, the DNA sequences of those old specimens are not sequenced successfully, but combining the features of morphology and geography, we conclude that *M. ovoidisporus* var. *angustatisporus* should be considered synonym of *M. broomeanus*.

Material examined: CHINA. SHANXI PROV.: Jincheng City, Yangcheng County, Sanglin Village, Shupigou, in soil under *Lonicera japonica* Thunb., Alt. 860 m, 27 August 1987, *M.-c. Chang* 242 (HMAS81917 ex MHSU2023); Linfen City, Xi County, Shenjiagou, in soil under *Quercus liaotungensis*, Alt. 1321 m, 10 September 2017, *Y.-y. Xu* 033 (BJTC FAN909); *ibid.*, in soil under *Pinus bungeana*, *X.-y. Yan* 155 (BJTC FAN1230); Yuncheng City, Yuanqu County, Lishan Village, 25 July 1987, *M.-c. Chang* 505 (HMAS83323 ex MHSU2002); *ibid.*, Alt. 1280 m, 8 August 1990, *Y. Ma*, *B. Qiao*, *W.-k. Bai* 502 (HMAS96692 ex MHSU2003). SICHUAN PROV.: Songpan County, Alt. 3100 m, 30 August 1991, *M.-s. Yuan* 1729 (HKAS24811).

Melanogaster fuisporus Y. Wang, in Wang, Chang, Tao & Liu, Journal of Shanxi University, Natural Science 18(4): 450 (1995) Fig. 3, g–j

Description

Basidiomata 1.1–1.6 × 1.2–3 cm, hypogeous, subglobose to irregularly globose, yellow brown to brown, without distinct basal zone; rhizomorphs few, brown; sweet odor, such as the banana flavor or the apple flavor. Gleba deep brown when fresh, black after dried, with sterile white veins scattered throughout. Peridium two-layered, epicutis 10–20 µm thick, composed of interwoven hyphae, light orange yellow, 4–5 µm in diam, smooth; subcutis 230–400 µm thick, composed of interwoven hyphae, with abundant inflated cells, irregular or puzzle-like, up to 5–40 µm wide, yellowish to pale orange yellow to ward outside surface; Trama plates of interwoven hyphae, hyaline to light yellow, thin-walled, 3–4 µm in diam, tending to be gelatinized. Basidia 9–12 × 10–23 µm, hyaline clavate-capitate when young, 2–3-spored, sterigmata short, collapsed and disappeared at maturity. Basidiospores fusiform, apex bluntly apiculate, 10.0–15.9 × 4.8–6.3 µm (avX = 12.3 ± 1.4 × 5.2 ± 0.3, n = 50), Q = 2.0–2.9 (avX = 2.3 ± 0.2, n = 50), orange brown or deep brown at maturity, 1 µm thick, smooth, the base broadly truncate-cupped, with very short remnants of sterigmata.

Habit, habitat and distribution: August to September, in soil under *Quercus monglica* Fish., *Q. acutissima* Carr. Northeast and South China.

Notes: *Melanogaster fuisporus* is re-described in details based on the re-examinations for paratypes cited by Wang et al. (1995) and newly added specimen HMAS83274 from type location. In this study, the beautiful illustrations were provided, intuitively presented the shape of the fusiform spore to people. The newly added specimen, HMAS83274 ex MHSU2008 used to be cited as *Melanogaster fuisporus* var. *obovatus* (this variety has been revised to *Melanogaster obovatus* based on present study.) in Flora Fungorum Sinicorum (Vol.7) (Liu et al. 1998). Although we sequenced unsuccessfully, we noticed this specimen was mature and fitted well with *M. fuisporus* in morphology, besides it was collected from type location in Chinese Yunnan Province. For the above reasons, we diagnosed this specimen as *M. fuisporus*. *Melanogaster fuisporus* is diagnosed by its fusiform basidiospores with a bluntly apiculate apex, orange brown or deep brown basidiospores at maturity, easy to be recognized. *Melanogaster quercus* differs from *M. fuisporus* by its brick red basidiomata and light yellowish basidiospores of 10.8–15.3 × 5.6–7.7 µm.

Material examined: CHINA. HEILONGJIANG PROV.: Ningan City, Jingpo Lake, in soil under *Quercus monglica*, 12 Sept 1987, *K. Tao*, *L. Fan* 193 (HMAS83095 ex MHSU2004, paratype); YUNNAN PROV.: Kunming City, Heilongtan, Kunming Botanical Garden, in soil under *Q. acutissima*, 10 August 1990, *M.-c. Chang*, *L. Wang*, *D.-s. Wang* 239 (HMAS96755 ex MHSU2005, paratype); *ibid.*, *M.-c. Chang*, *L. Wang* 224 (HMAS83274 ex MHSU2008).

Melanogaster minobovatus L. Fan & X.Y. Yan *sp.nov.* MycoBank MB Fig. 3, k–n

Etymology: *minobovatus*, *min*, minor, *obovatus*, obovoid, referring to the size and shape of basidiospores.

Type: CHINA. SHANXI PROV.: Linfen City, Xi County, shenjiagou, in soil under *Pinus bungeana*, Alt. 1321 m, 10 Sept 2017, *Y.-y. Xu* 035 (BJTC FAN911).

Description

Basidioma 3.5 × 4.5 cm, hypogeous, subglobose, yellow-brown, without distinct basal zone; rhizomorphs invisible, Strong wine aroma or fruit aroma. Gleba black, liquefying to produce a very dark spore mass. Peridium two-layered, 20–50 µm thick, composed of interwoven hyphae, 4–7 µm in diam, smooth, clamp connections abundant; subcutis 300–450 µm thick, composed of interwoven hyphae, with a large number of inflated cells, ellipsoidal, irregular or puzzle-like, up to 6.5–17 µm wide, light yellowish to yellow toward outside surface. Trama plates of hyaline gelatinized hyphae. Basidia not observed. Basidiospores obovoid, 5.1–6.9 × 4.2–5.1 µm (avX = 6.1 ± 0.4 × 4.7 ± 0.2, n = 50), Q = 1.1–1.5 (avX = 1.3 ± 0.1, n = 50), deep brown at maturity, 0.5–1 µm thick, smooth, the base truncate-cupped, with very short remnants of sterigmata.

Habit, habitat and distribution: September, in soil under *Pinus bungeana*. Warm temperate zone of North China.

Notes

This species is distinguished from other *Melanogaster* species by its basidioma with strong wine aroma or fruit aroma, and small, obovoid and deep brown basidiospores. *Melanogaster minisporus* Cázares, Guevara, described from Mexico by Cázares et al. (2008), is similar to *M. minobovatus* in basidiospore shape and size. However, *M. minisporus* differs from *M. minobovatus* in its bright yellow basidiomata and dark brown gleba with white or yellow at maturity. Phylogenetically, an environmental sequence (Genbank accession JQ318611) associated with *Quercus liaotungensis* from Loess Plateau, China was grouped a strong support clade (BS = 100, PP = 1.00) together with *M. minobovatus* (Fig. 1), and both of them shared 99% ITS similarity from each other, which supported that *M. minobovatus* is a distinct species, and is associated to *Quercus* sp. and *Pinus* sp.

Melanogaster obovatus (K. Tao, Ming C. Chang & B. Liu) L. Fan & X.Y. Yan *comb. & stat. nov.* MycoBank MB Fig. 3, o-r

≡ *Melanogaster fusisporus* var. *obovatus* K. Tao, Ming C. Chang & B. Liu, Journal of Shanxi University, Natural Science 18 (4): 450 (1995). Type:—CHINA. SHANXI PROV.: Yucheng City, Yuanqu County, Lishan Town, Houwentang Village, in soil under *Spiraea pubescens* Turcz., 2 August 1990, *M.-c. Chang* 193 (HMAS83257 ex MHSU1470).

Diagnosis: *Melanogaster obovatus* is diagnosed by its light brick red or orange brown basidiomata, yellow green to yellow brown gleba, elongate-fusiform with rounded top to obovate and light yellow brown basidiospores. *Melanogaster fusisporus* differs from *M. obovatus* by its black gleba at maturity, fusiform with acute apex and dark brown basidiospores of (10–)11.5–13(–15) × (5–)5.5–6(–6.5) µm. *Melanogaster intermedius* (Berkeley) Zeller & Dodge, described from England and America, is somewhat similar to *M. obovatus* in basidiomata appearance. However, *M. intermedius* is distinguished from *M. obovatus* by its thin peridium of 125–160 µm, slaty black gleba and wide basidiospores of 11–13 × 7.4–8 µm.

Description

Basidiomata 1.5–2.3 × 0.7–1.5 cm, hypogeous, subglobose, light brick red or orange brown, minutely tomentose, without distinct basal zone; rhizomorphs few, brown; sweet odor, like fruits flavor. Gleba gelatinous, yellow green to yellow brown, with sterile white veins scattered throughout, liquefying to become the slimy spore mass at maturity. Peridium two-layered, epicutis 50–100 µm thick, composed of interwoven hyphae, yellow brown, 4–10 µm in diam, smooth, clamp connections abundant; subcutis 300–500 µm thick, composed of interwoven hyphae, with massive inflated cells, ellipsoidal, irregular, up to 10–32 µm wide, yellowish to yellow brown toward outside surface. Trama plates of hyaline or yellowish gelatinized hyphae. Basidia 10–14.2 × 4.9–6.7 µm, light yellow clavate-capitate when young, 4–spored, sterigmata short, collapsed and disappeared at maturity. Basidiospores elongate-fusiform with rounded top to obovate, 10.3–14.8 × 4.6–7.3 µm (avX = 12.2 ± 0.9 × 6.0 ± 0.4, n = 50), Q = 1.7–2.6 (avQ = 2.1 ± 0.2, n = 50), light yellow brown, walls 0.5 µm thick, smooth, the base broadly truncate-cupped, with very short remnants of sterigmata.

Habit, habitat and distribution

June to September, in soil under *Quercus* sp., *Pinus tabuliformis* Carr. Temperate zone of North and Northeast China.

Notes

Phylogenetic analysis based on the sequences of specimens newly collected from type locality and morphologically consistent with the type specimen of *M. fusisporus* var. *obovatus*, revealed that these sequences clustered the independent clade J with a strong support (BS = 96/PP = 1.00) (Fig. 1), supporting they represented a distinct species. Therefore, we raised *M. fusisporus* var. *obovatus* to species level as *M. obovatus*, and an epitype (BJTC FAN1091) was designated here. Phylogenetically, *M. quercus* and *M. shanxiensis* were related to *M. obovatus* closely. However, they morphologically differed from *M. obovatus* in black gleba and elongate-fusiform spores with apiculate apex for the former and white to pale yellow basidioma and elongate-obovatus basidiospores for the latter.

Material examined: CHINA. JILIN PROV.: Dunhua city, Dashitou county, under *Quercus mongolica* Fisch. ex Ledeb., 15 September 1987, L. Fan, K. Tao 226 (HMAS83269 ex MHSU2010). SHANXI PROV.: Jinzhong city, Lingshi county, Houhuigou, 27 June 1987, under *Q. liaotungensis* Koidz., T.-I. Shangguan 234 (HMAS81918 ex MHSU2015); Yuncheng City, Yuanqu county, Lishan Town, Houwentang Village, Alt. 1280 m, 2 August 1990, Y. Ma, B. Qiao, X.-k. Bai 198 (HMAS83094 ex MHSU2006); *ibid.*, X.-k. Bai, B. Qiao, Y. Ma 215 (HMAS83267 ex MHSU2007); *ibid.*, Y. Ma, B. Qiao, X.-k. Bai 225 (HMAS83265 ex MHSU2009); *ibid.*, Y. Ma, X.-k. Bai, B. Qiao 227 (HMAS83266 ex MHSU2011); *ibid.*, B. Qiao, Y. Ma, X.-k. Bai 501 (HMAS83268 ex MHSU2018); *ibid.*, 1 August 1990, Y. Ma, X.-k. Bai, B. Qiao 231 (HMAS83270 ex MHSU2014); *ibid.*, Y. Ma, B. Qiao, X.-k. Bai 503 (HMAS83272 ex MHSU2019); *ibid.*, under *Q. liaotungensis*, 27 July 1987,

M.-c. Chang 228 (HMAS83271 ex MHSU2012); *ibid.*, Alt. 1900 m, 24 July 1987, *M.-c. Chang* 230 (HMAS83264 ex MHSU2013); *ibid.*, 20 July 1987, *M.-c. Chang, Y.-h. Chang* 240 (HMAS83273 ex MHSU2017); *ibid.*, 25 July 1987, *M.-c. Chang* 236 (HMAS83275 ex MHSU2016); Yuncheng City, Xia County, Sijiao Town, Yujialing Village, Alt. 970 m, 27 October 2017, in soil under *Q. variabilis*, *T. Li* 055 (BJTC FAN1081); *ibid.*, *X.-y. Yan* 123 (BJTC FAN1091, epitype, designated here); in soil under *Q. variabilis* Bl., *Y.-y. Xu* 072 (BJTC FAN1102-A); *ibid.*, *Y.-y. Xu* 072 (BJTC FAN1102-B); *ibid.*, in soil under *Pinus tabuliformis*, *K.-b. Huang* 156 (BJTC FAN1118); *ibid.*, 19 August 2019, in soil under *Quercus* sp., *Y.-y. Xu* 150 (BJTC FAN1208).

Melanogaster panzhihuaensis L. Fan & X.Y. Yan *sp.nov.* MycoBank MB Fig. 3, s–v

Etymology. *panzhihuaensis*, referring to the type locality, where the type was collected.

Type. CHINA. SICHUAN PROV.: Panzhihua City, in soil under *Pinus yunnanensis* Franch., 1995, *Z.-j. Gu* 6 (HMAS 81915 ex MHSU2266).

Diagnosis. *Melanogaster panzhihuaensis* is diagnosed by its oval basidiospores with blunt and narrow apex and reddish brown basidiospores at maturity.

Description. *Basidioma* 1.5 × 1.7 cm, hypogeous, subglobose, brown to dark brown when dry; no rhizomorphs visible; odor no record. Gleba pale yellow-brown when fresh, firm, black after dried, with sterile white veins scattered throughout. Peridium single-layered, 150–220 µm thick, compactly interwoven hyphae, 2.5–5 µm, with a large number of inflated cells, puzzle-like or irregular, up to 7.5–13 µm wide, light yellowish to yellow walls toward surface. Trama plates of interwoven hyphae, pale orange–yellow, thin-walled, 2–5 µm in diam. Basidia not observed. Basidiospores oval, apex blunt and narrow, 8.7–12.4 × 5.2–7.6 µm (avX = 10.4 ± 0.8 × 6.1 ± 0.6, n = 45), Q = 1.4–2.0 (avX = 1.7 ± 0.2, n = 45), reddish brown, the base truncate-cupped, walls 1 µm thick, smooth, the base broadly truncate-cupped, with very short remnants of sterigmata.

Habit, habitat and distribution

in soil under *Pinus yunnanensis*, Southwest China.

Notes

In present study, the sequence of *M. panzhihuaensis* showed less than 89.89% ITS similarity to other species and formed an independent clade U (Fig. 1), indicating it was a distinct species. In addition, the sequences of *M. minobvatus* formed sister clade with *M. panzhihuaensis*. However, *M. minobvatus* is differentiated from *M. panzhihuaensis* by its yellow-brown basidiomata, obovoid and small basidiospores of 5.1–6.9 × 4.2–5.1 µm. Morphologically, *Melanogaster natsii* is similar to *M. panzhihuaensis* in the color of gleba, but *M. natsii* differs from the latter in its broadly citriform or obovatus and pale yellow brown basidiospores.

Melanogaster quercus L. Fan & X.Y. Yan *sp.nov.* MycoBank MB Fig. 4, a–d

Etymology. *quercus*, referring to the host of this species.

Type. CHINA. SHANXI PROV.: Lvliang City, Jiaocheng County, Panguangou, in soil under *Quercus* sp., Alt. 1879 m, 6 September 2017, *Y.-y. Xu* 011 (BJTC FAN809).

Diagnosis. *Melanogaster quercus* is diagnosed by brick red appearance, black gleba and light yellow elongate-fusiform basidiospores with apiculate apex. *M. obovatus* is closely related to *M. quercus*. However, *M. obovatus* differs from *M. quercus* in its yellow green to yellow brown gleba and elongate-fusiform with rounded top to obovate spores.

Description. *Basidiomata* 1.8–2 × 2–2.5 cm, hypogeous, subglobose, brick red, without distinct basal zone; no rhizomorphs visible; slightly sweet odor. Gleba gelatinous-firm, black, irregular glebal chambers separated by sterile white veins. Peridium two-layered, epicutis 50–100 µm thick, composed of interwoven hyphae, yellow, 4.5–10 µm in diam, clamp connections visible; subcutis 400–500 µm thick, composed of interwoven hyphae, with massive inflated cells, ellipsoidal, puzzle-like or irregular, 10–50 µm, thin-walled, yellowish to yellow toward outside surface. Trama plates of hyaline or yellowish gelatinized hyphae. Basidia not observed. Basidiospores elongate-fusiform, apex apiculate, 10.8–15.3 × 5.6–7.7 µm (avX = 13.1 ± 1.1 × 6.5 ± 0.5, n = 50), Q = 1.6–2.4 (avX = 2.0 ± 0.2, n = 50), light yellowish, 0.5 µm thick, smooth, the base truncate-cupped, with very short remnants of sterigmata.

Habit, habitat and distribution

September, in soil under *Quercus* sp. Warm temperate zone of North China.

Notes

Phylogenetically, the sequences of *Melanogaster quercus* clustered together and branched as the sister group to the sequence of *M. shanxiensis* with no support value, implying that *M. quercus* is a distinct species and related to *M. shanxiensis*. However, *M. shanxiensis* differs from *M. quercus* in its white to pale yellow basidiomata and elongate-obovate basidiospores. Morphologically and phylogenetically, *M. obovatus* is closely related to *M. quercus*. However, *M. obovatus* differs from *M. quercus* in its yellow green to yellow brown gleba and elongate-fusiform with rounded top to obovate spores. In addition, the

sequences of the two species were grouped in two different branches (I and J) with a high support value (BS = 95/PP = 0.95) and (BS = 99/PP = 1.00) respectively.

Material examined. CHINA. SHANXI PROV.: Lvliang City, Jiaocheng County, Pangquangou, in soil under *Quercus* sp., Alt. 1879 m, 6 September 2017, Y.-y. Xu 010 (BJTC FAN808); *ibid.*, in soil under *Q. liaotungensis*, T. Li 015 (BJTC FAN832).

Melanogaster shanxiensis B. Liu, K. Tao & Ming C. Chang, Acta Mycol. Sin. 8(3): 210 (1989) Fig. 4, e–h

Description. Basidiomata 2–2.9 × 1.5–1.8 cm, hypogeous, subglobose, white to pale yellow when fresh, without distinct basal zone, smooth to minutely tomentose when dry; rhizomorphs visible; odor no record. Gleba firm, pale brown when young to brown to brownish black when mature, irregular glebal chambers separated by sterile yellowish brown veins. Peridium two-layered, epicutis 30–70 µm thick, composed of interwoven hyphae, deeply orange-yellow, 5.0–5.5 µm in diam, clamp connections visible; subcutis 150–350 µm thick, composed of interwoven hyphae, with massive inflated cells, ellipsoidal to irregular, 7–25 µm, thin-walled, yellowish to yellow toward outside surface. Trama plates of hyaline or yellowish gelatinized hyphae. Basidia not observed. Basidiospores elongate-obovate to narrowly oblong, apex obtuse, 10.5–14.5 × 5.0–6.5 µm (avX = 12.2 ± 1.0 × 6.1 ± 0.4, n = 50), Q = 1.6–2.3 (avX = 2.0 ± 0.2, n = 50), light yellow brown, 1 µm thick, smooth, the base truncate-cupped, with very short remnants of sterigmata.

Habit, habitat and distribution

September, in soil under *Lespedeza bicolor* Turcz. Warm temperate zone of North China.

Notes

Melanogaster shanxiensis can be easily distinguished from other *Melanogaster* species by its white to pale yellow basidiomata and yellow brown elongate-obovate to narrowly oblong basidiospores 10.5–14.5 × 5.0–6.5 µm. In present study, the sequence newly obtained from holotype (HMAS81910) of *Melanogaster shanxiensis* showed less than 97.91% ITS similarity to other species, indicating it is a distinct species. Phylogenetic analysis reveals this sequence is sister clustered with *M. quercus* with no support value, implying that *M. shanxiensis* is a distinct species and related to *M. quercus*. Morphologically, *M. quercus* are differentiated from *M. shanxiensis* by brick red basidiomata and elongate-fusiform basidiospores.

Material examined. CHINA. SHANXI PROV.: Yuncheng City, Yuanqu County, Lishan township, in soil under *Lespedeza bicolor* Turcz., 10 September 1987, M.-c. Chang 197 (HMAS81910 ex MHSU909, Holotype).

Melanogaster spinisporus Y. Wang, in Wang, Chang, Tao & Liu, Journal of Shanxi University, Natural Science 18(4): 450 (1995), emend. L. FAN & X.Y. Yan Fig. 4, i–n

Description

Basidiomata 3–3.5 × 4–5 cm, hypogeous, sometimes exposed, subglobose, light brown with some red brown or brick red when fresh, light brown to brown when dry, without distinct basal zone; rhizomorphs few, brown; strong wine aroma. Gleba dark red-brown to black when fresh, firm, gelatinous, with sterile white veins scattered throughout, trend toward liquefaction, black and hard when dry. Peridium single-layered, 360–750 µm thick; compactly interwoven hyphae, 5–7.5 µm in diam, thin-walled, light yellowish or yellow, clamp connections abundant. Trama plates of gelatinized hyphae. Basidia 23.0–36.5 × 8.0–14.5 µm, light brown clavate-capitate when young, 4-spored, sterigmata short, collapsed and disappeared at maturity. Basidiospores broadly ellipsoid to obovoid, subglobose, bilaterally symmetric, appearing smooth or decorated with sparse spine under light microscope (LM), 0.5–1 µm high, short strip, even connected into small pieces, irregularly verrucose under scanning electron microscope (SEM); 7.4–12.0 × 5.2–9.2 µm (avX = 9.6 ± 1.0 × 7.3 ± 0.7, n = 50), Q = 1.1–1.7 (avX = 1.3 ± 0.1, n = 50), deep brown at maturity, walls 0.5–1 µm thick the base truncate-cupped, with very short remnants of sterigmata.

Habit, habitat and distribution

Summer and Autumn (July, September to October), in soil under *Quercus* sp, *Pinus bungeana*, sometimes exposed. Warm temperate zone of North China.

Notes. *Melanogaster spinisporus* is diagnosed by its light brown with some red brown or brick red tints basidiomata, broadly ellipsoid to obovoid or subglobose basidiospores, decorated with short strip, even connected into small pieces, irregularly verrucose under SEM. *Melanogaster spinisporus* was original described obovoid spore, spinose and spine 0.5 µm high by Wang et al. (1995). However, we checked the holotype of *Melanogaster spinisporus* (HMAS27263 collected from Beijing of China in 1958 by Shuqun Deng), confirmed the spore ornamented by verrucose under SEM, but failed to obtain sequence. Phylogenetic analysis based on the sequences of specimens (BJTC FAN936, BJTC FAN938, BJTC FAN941, BJTC FAN1085, BJTC FAN1092) newly collected from Shanxi Province and morphologically consistent with the type specimen of *M. spinisporus* revealed that these sequences clustered in independent clade H with a strong support (BS = 99/PP = 1.00), supporting they represented a distinct species (Fig. 1). Therefore we recognized it as *M. spinisporus*, emended the description and an epitype (BJTC FAN941-A) was designated here. Phylogenetically, *M. obovatus*, *M. quercus*, *M. shanxiensis*, *M. tomentellus* were related to *M. spinisporus*. However, the formers could be distinguished obviously from the latter by their smooth elongate-fusiform or elongate-obovate spores. Morphologically, *Melanogaster utriculatus* Y. Wang, Castellano & Trappe have obovatus basidiospores with decoration described by Wang et al. (2005) from Japan is similar to *M. spinisporus*. However, *M. utriculatus* differs from *M. spinisporus* by its bigger spores (9–)11–15(–17) × (7–)8–11 µm and appressed or inflated utricle on the spore surface.

Material examined: CHINA. BEIJING: Beijing City, Mentougou District, Tantuo Temple, on the ground of *Quercus* sp. forest, July 1958, *S.-q. Teng* 4809 (HMAS27263, Holotype). SHANXI PROV.: Linfen City, Xi County, shenjiagou, in soil under *Pinus bungeana*, *Alt.* 1321 m, 10 September 2017, *K.-b. Huang* 110 (BJTC FAN936-A); *ibid.*, *K.-b. Huang* 110 (BJTC FAN936-B); *ibid.*, *K.-b. Huang* 110 (BJTC FAN936-C); *ibid.*, *Alt.* 1326 m, in soil under *Pinus bungeana*, *K.-b. Huang* 112 (BJTC FAN938); *ibid.*, *K.-b. Huang* 115 (BJTC FAN941-A, epitype, designated here); *ibid.*, *K.-b. Huang* 115 (BJTC FAN941-B); Yuncheng City, Xia County, Sijiao Town, Yujialing Village, in soil under *Quercus variabilis*, *Alt.* 970 m, 27 October 2017, *T. Li* 059 (BJTC FAN1085); *ibid.*, *X.-y. Yan* 124 (BJTC FAN1092).

Melanogaster subglobisporus K. Tao, Ming C. Chang & B. Liu, in Wang, Chang, Tao & Liu, *Journal of Shanxi University, Natural Science* 18(4): 451 (1995), emend. L. Fan & X.Y. Yan

Description

Basidiomata 1.5–2 × 0.5–1.5 cm, hypogeous, subglobose, yellow brown to brown when fresh, rust brown to deep brown when dry; rhizomorphs few, brown; sweet odor, like fruits flavor. Gleba firm, black, with sterile white veins scattered throughout. Peridium two-layered, epicutis 60–100 µm thick, composed of interwoven hyphae, yellow brown, 5–7.5 µm in diam, clamp connections visible; subcutis 100–200 µm thick, compactly interwoven hyphae, with some inflated cells, ellipsoidal, irregular, up to 7.5–15 µm wide, light yellowish to yellowish brown walls toward surface. Trama plates of interwoven hyphae, hyaline to pale yellow, 2.5–4 µm in diam. Basidia not observed. Basidiospores obovate, appearing smooth under LM, irregularly tiny verrucose under SEM, 8.7–10.6 × 6.6–8.0 µm (avX = 9.8 ± 0.5 × 7.0 ± 0.4, n = 50), Q = 1.1–1.5 (avX = 1.4 ± 0.1, n = 50), yellowish brown to brown, walls 1 µm thick.

Habit, habitat and distribution

Summer (July), in soil under *Quercus* sp. Warm temperate zone of North China.

Notes: *Melanogaster subglobisporus* is diagnosed by its yellow brown to brown basidiomata and obovate basidiospores decorated with irregularly tiny verrucose under SEM. *Melanogaster subglobisporus* has obovoid or subglobose and smooth basidiospores in original descriptions (Wang et al. 1995). However, after checking the holotype and two specimens of *M. subglobisporus* (collected from type location) cited in *Flora Fungorum Sinicorum* (Vol. 7) (Liu et al., 1998), we confirmed its spores were ornamented with irregularly tiny verrucose under SEM. Phylogenetically, the sequence of holotype (HMAS83329 ex MHSU2001) clustered in the independent clade M and showed less than 91.49% ITS similarity with other species. Therefore we recognized the phylogenetic position of *M. subglobisporus* and emended the description with illustration. Phylogenetically and Morphologically, *M. spinisporus* is related to *M. subglobisporus* with similar spore shape and size. However, *M. spinisporus* differs from *M. subglobisporus* by its light brown with some red brown or brick red basidiomata and basidiospore ornamented by short strip, even connected into small pieces, irregularly verrucose under SEM.

Material examined: CHINA. SHANXI PROV.: Jincheng City, Yangcheng County, Yangzhuang river, *Alt.* 580–600 m, 16 July 1990, *Y. Ma, M.-c. Chang* 504 (HMAS83329 ex MHSU2001, Holotype); *ibid.*, *Y. Ma, M.-c. Chang* 243 (HMAS81919 ex MHSU2107, epitype, designated here); *ibid.*, *Alt.* 600–620 m, under *Quercus* sp., 13 July 1990, *M.-c. Chang* 500 (HMAS81921 ex MHSU2110).

Melanogaster tomentellus L. Fan & X.Y. Yan *sp. nov.* MycoBank MB Fig. 4, s–w

Etymology: *tomentellus*, referring to the tomentose surface of the basidiomata.

Type: CHINA. YUNNAN PROV.: Kunming City, Xishan District, Haikou Town, in soil under *Pinus* sp., *Alt.* 1900 m, 8 January 2016, *J.-z. Cao* 1707 (BJTC FAN539).

Diagnosis: *Melanogaster tomentellus* could be easily diagnosed by its minutely green tomentose surface of basidiomata, small and elongate-fusiform spores.

Description

Basidiomata 1.1–2.2 × 1.9–2.5 cm, hypogeous, globose to subglobose, brown to deep brown, surface minutely green tomentose, without distinct basal zone, few brown rhizomorphs; odor strong turpentine flavor. Gleba black, solid, gelatinous, with sterile white veins scattered throughout. Peridium single-layered, 250–350 µm thick, textura globosa to textura prismatica structure (pseudoparenchymatous), made of short, subglobose, ellipsoidal or irregular round hyphae, 5–30 µm, thin-walled, light yellowish to yellow brown walls toward outside surface. Cystidia up to 15–40 µm long and 5–7.5 µm broad at base arising from some of the outermost cells of the peridium, clamp connections visible, thick-walled at apical tips. Trama composed of gelatinized hyphae. Basidia not observed. Basidiospores elongate fusiform, 8–11.4 × 3.8–5.6 µm (avX = 9.4 ± 0.8 × 4.5 ± 0.4, n = 50), Q = 1.7–2.7 (avX = 2.1 ± 0.2, n = 50), brown, walls 0.5 µm thick, smooth, the base broadly truncate-cupped, with very short remnants of sterigmata.

Habit, habitat and distribution

Winter (January), in soil under *Pinus* sp., plateau subtropical evergreen forests of Southern China.

Notes

Phylogenetically, the sequence of holotype (BJTC FAN539) clustered in the independent clade L with strong support (BS = 97/PP = 1.00) and showed less than 95.33% ITS similarity with other species. Therefore, we proposed *M. tomentellus* as a new species from China. *M. obovatus*, *M. quercus* and *M. shanxiensis*

are related to *M. tomentellus* phylogenetically. However, *M. obovatus* and *M. quercus* differ from *M. tomentellus* in their light brick red or orange brown surface and yellowish or yellow brown basidiospores. *M. shanxiensis* differs from *M. tomentellus* in its white to pale yellow surface, yellow brown and big basidiospores (10.5–14.5 × 5.0–6.5 μm).

Key to species of *Melanogaster* from China

1. Basidiospores verrucose.....2

1. Basidiospores smooth.....3

2. Basidiospores appearing smooth or decorated with sparse spine under light microscope (LM), but short strip, even connected into small pieces, irregularly verrucose under scanning electron micro-scope (SEM), 7.4–12.0 × 5.2–9.2 μm.....*M. spinisporus*

2. Basidiospores appearing smooth under LM, irregularly tiny verrucose under SEM, 8.7–10.6 × 6.6–8.0 μm.....*M. subglobisporus*

3. Basidiospores light yellow to yellow brown.....4

3. Basidiospores brown to deep brown or reddish brown.....7

4. Basidiospores ovoid to ellipsoid, small, 5–7 μm long*M. ovoidisporus*

4. Basidiospores broadly citriform or obovatus to fusiform, large, 9.5–16 μm long,5

5. Basidiospores elongate-obovatus to narrowly oblong, 10.5–14.5 × 5.0–6.5 μm.....*M. shanxiensis*

5. Basidiospores broadly citriform or obovatus, 9.6–13.3 × 4.8–9.8 μm.....*M. natsii*

5. Basidiospores elongate-fusiform, 10–15 × 4.6–7.7 μm.....6

6. Gleba yellow green to yellow brown, basidiospores elongate-fusiform with rounded top, or obovate*M. obovatus*

6. Gleba black, basidiospores elongate-fusiform with apiculate apex*M. quercus*

7. Basidiospores reddish brown.....*M. panzhihuaensis*

7. Basidiospores yellow brown to deep brown.....8

8. Basidiospores ellipsoid, oblong to cylindrical, 4.4–7.8 × 3.3–4.7 μm.....*M. broomeanus*

8. Basidiospores fusiform.....9
8. Basidiospores obovoid.....10
9. Basidiospores fusiform, apex bluntly apiculate, 10.0–15.9 × 4.8–6.3 μm.....*M. fusisporus*
9. Basidiospores elongate fusiform, apex apiculate, 8–11.4 × 3.8–5.6 μm.....*M. tomentellus*
10. Gleba yellowish to brown, basidiospores obovoid, sometimes narrowly oblong or ellipsoid, 6.5–8 × 3.8–4.7 μm.....*M. obovatisporus*
10. Gleba black, basidiospores obovoid, 5.1–6.9 × 4.2–5.1 μm.....*M. minobovatus*

Discussion

The circumscription and diversity of *Melanogaster*

Totally 21 phlotypes were defined based on ITS phylogeny (Fig. 1), of which 9 phlotypes were recognized as known species, including *M. intermedius*, *M. ambiguus*, *M. eurypermus*, *M. spinisporus*, *M. shanxiensis*, *M. subglobisporus*, *M. rivularis*, *M. luteus* and *M. broomeanus*; 5 phlotypes were described as 4 new species and 1 new combination based on the Chinese collections in this present study. The remaining 7 phlotypes were most likely new species of the genus *Melanogaster*. Moreover, it should be mentioned that there were still 15 species not to be included in the present molecular analysis among the about 25 species accepted in *Melanogaster* before this study, including *Melanogaster carolinensis* (Bosc ex Spreng.) De Toni, *Melanogaster cauvinius* (Corda) Mont. ex Berl., De Toni & E. Fisch., *Melanogaster durissimus* Cooke, *Melanogaster eisenii* Harkn., *Melanogaster macrocarpus* Zeller, *Melanogaster minisporus* Cázares, G. Guevara, J. García & Trappe, *Melanogaster natsii*, *Melanogaster ovoidisporus*, *Melanogaster parksii* Zeller & C.W. Dodge, *Melanogaster trappei* I.P.S. Thind & B.M. Sharma, *Melanogaster tuberiformis* Corda, *Melanogaster umbrinigliebus* Trappe & Guzmán, *Melanogaster utriculatus*, *Melanogaster variegates* and *Melanogaster vittadinii* Soehner & Knapp. These putative novel species and old species without DNA data required more mycologists to pay attention to them in the future. Morphologically, the spore shape and size of a species is basically regular and stable, which is usually used in the species delimitation of *Melanogaster*, but all in all, the combination of spore characteristics, peridium structure, color of basidiomata and gleba, can perfectly help us distinguish species. For example, *M. obovatus* and *M. quercus* have similar spore shape and size, the gleba, however, is yellow green to yellow brown in *M. obovatus*, but black in *M. quercus*, by which the two species are completely differentiated from each other.

DNA analyses revealed that a threshold of 98% identity in ITS sequence could be also used as the delimitation of *Melanogaster* species. Most of species delimited in this study share less than 98% ITS similarity from each other. Furthermore, combined our result with previous published data, we basically proposed the main diagnostic characteristics for the genus *Melanogaster*. Basidiomata without distinct basal zone; Peridium single or two-layered, composed of interwoven hyphae or textura globosa to texturaprimatica structure (pseudoparenchymatous), clamp connections visible usually; Gleba gelatinous, with sterile veins scattered throughout, basidia clavate, 2–8-spored, which were not regularly arranged in a definite palisade, but irregularly distributed through broad hyphal zone lining the cavities; Basidiospores ellipsoid, subglobose, fusiform or lemon shaped, most deeply colored, some light colored, most smooth, a few decorated with verrucose or utricle.

According to the literatures from Europe (Berkeley, 1844; Moreno et al. 1986; Ainsworth, 2005; Lacheva, 2015), North America (Harkness, 1899), Asia (Liu et al, 1989; Wang et al. 1995, 2005), and Oceania (Cunningham et al, 1934), the genus *Melanogaster* form ectomycorrhizae with a wide range of host plant, including: *Acer* sp., *Alnus* sp., *A. alnobetula* (Ehrh.) K.Koch, *A. cordata* (Loisel.) Duby, *Corylus avellana* L., *Fagus* sp., *F. sylvatica* L., *Lithocarpus densiflorus* (Hook. & Arn.) Rehder, *Lonicera japonica*, *Pinus sylvestris* L., *Populus nigra* L., *Pseudotsuga menziesii* (Mirb.) Franco, *Quercus* sp., *Q. liaotungensis*, *Spiraea pubescens*, *Tsuga* sp. In China, according to our present research, the host included *Lonicera japonica*, *Ostryopsis davidiana*, *Pinus bungeana*, *Pinus tabuliformis*, *Quercus acutissima*, *Quercus mongolica*, *Quercus liaotungensis*, *Spiraea pubescens*. In conclusion, the *Melanogaster* species mainly establish symbiosis with plants of *Fagaceae*, *Pinaceae*, and *Betulaceae*, which may be the reason why the genus *Melanogaster* is widely distributed.

Additionally, our phylogenetic analyses revealed that some samples labeled as "*Alpova trappei*" were grouped into the *Melanogaster* clade (Fig. 1, 2), indicating they probably represented the species of *Melanogaster* rather than that of *Alpova*. However, whether these samples were misidentification or the species "*Alpova trappei*" should be transfer to *Melanogaster* need further research.

Melanogaster in China

Up to now, there are ten species and varieties to be described from China based on morphological data alone according to our data. In the present study the collections of *Melanogaster* from China, including the old specimens deposited in HMAS and KUN-HKAS, and the ones recently collected by us, were researched using morphological and molecular phylogenetic methods. Consequently, the occurrence of four known species in China was confirmed, including *M. broomeanus*, *M. shanxiensis*, *M. spinisporus*, *M. subglobisporus*. The variety *M. ovoidisporus* var. *angustatisporus* was treated as synonym of *M. broomeanus*, while *M. fusisporus* var. *obvatus* was raised to species level as *M. obvatus*. DNA sequences of the other four species, *M. fusisporus*, *M. obovatisporus*, *M. ovoidisporus* and *M. natsii*, were not successfully sequenced in this study because the type specimens and reliable specimens cited in *Flora Fungorum Sinicorum* (Vol. 7) (Liu et al., 1998) are old or poorly preserved, but they have distinct morphological features according to our reexaminations for their type specimens and reliable specimens. We therefore confirmed their occurrence in China. Moreover, four new species were proposed from China based on the newly collected specimens, including *M. minobovatus*, *M. panzhihuaensis*, *M. quercus* and *M. tomentellus*.

Totally, the occurrence of 13 *Melanogaster* species in China were confirmed in present study. In addition, there are still potential *Melanogaster* species that have not been discovered in China, such as the sequence of ECM (HM105535), which should represent a potential new taxon (Phylotype R: labeled as *M.sp.6*, Fig. 1) from China.

Conclusions

ITS phylogenetic analysis for the genus *Melanogaster* showed 21 phylogenetic species. Thirteen species were found from China including 8 known species, 4 species and one new combination. Their descriptions, illustrations and key were provided. This work is the first detailed morphological and phylogenetic analysis for the Chinese *Melanogaster* species, which would contribute to future research on *Melanogaster*.

Abbreviations

LM: light microscope; SEM: scanning electron microscope; ML: maximum likelihood BS: maximum PP: Bayesian posterior probabilities; HMAS: Herbarium Mycologicum Academiae Sinicae, Institute of Microbiology, Chinese Academy of Sciences; KUN: Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences; ITS: Ribosomal DNA internal transcribed spacer region; nrLSU: nuclear ribosomal large subunit.

Declarations

Ethics approval and consent to participate

Not applicable

Adherence to national and international regulations

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Alignments and tree files generated during the current study are available in the TreeBASE (accession <https://www.treebase.org/treebase-web/home.html>; study ??). All sequence data are available in NCBI Genbank following the accession numbers in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XY wrote the manuscript, made a substantial contribution to the conception of the study, interpreted the sequence data and performed the phylogenetic analysis and microscopy. LF contributed to microscopic examination of fungal specimens, advised on the interpretation of the taxonomy, and was a major contributor in writing the manuscript. YX and TL assisted in the analyses of the data and advised on the interpretation of the taxonomy. TZ and JL contributed to the collection of fungal specimens and made a substantial contribution to the conception of the study. All the authors read and approved the final manuscript.

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References

1. Ainsworth M (2005) *Melanogaster intermedius* a Slime Truffle rediscovered in England after 159 years. *Field Mycology* Volume 6(4). doi:10.1016/s1468-1641(10)60331-3
2. Alfaro ME, Zoller S, Lutzoni F (2003) Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution* 20:255–266. doi: 10.1093/molbev/msg028
3. Altschul S, Madden T, Schaffer A et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25:3389–3402 doi:10.1093/nar/25.17.3389
4. Binder M, Hibbett DS (2006) Molecular Systematics and Biological Diversification of *Boletales*. *Mycologia* 98(6):971-981. doi:10.1080/15572536.2006.11832626
5. Bonito GM, Gryganskyi AP, Trappe JM, Vilgalys R (2010) A global meta-analysis of Tuber ITS rDNA sequences: species diversity, host associations and long-distance dispersal. *Molecular Ecology* 19(22):4994–5008. doi: 10.1111/j.1365-294X.2010.04855.x
6. Brock PM, Doring H, Bidartondo MI (2009) How to know unknown fungi: the role of a herbarium. *New Phytol.* 181(3),719-724. doi:10.1111/j.1469-8137.2008.02703.x
7. Castresana J (2000) Selection of conserved blocks from multiple alignments and their use in phylogenetic analysis. *Molecular Biology and Evolution* 17:540–552. doi:10.1093/oxfordjournals.molbev.a026334
8. Corda AKJ (1831) Die Pilze Deutschlands. In: Sturm J. (ed.), Deutschlands Flora 3rd ed 3(12):1-36.
9. Dring DM (1971) Techniques for microscopic preparation. *Methods in microbiology*. Vol. 4. New York: Academic Press. p. 95–111.
10. Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113-118. doi:10.1111/j.1365-294X.1993.tb00005.x
11. Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42:182–192. doi:10.2307/2992540
12. Keissler K, Lohweg H (1937) *Fungi in Handel-Mazzetti Symbolae Sinicae*. 2: 37-66.
13. Lacheva M (2015) New records of *Melanogaster* species (Fungi, *Boletales*) in Bulgaria. *Journal of Biodiversity and Environmental Sciences* 6:494-500.
14. Liu B, Tao K, Cang MC (1989) Two new species of *Melanogaster* from China. *Acta Mycologia Sinica* 8(3):210-213.
15. Moreau PA, Rochet J, Richard F, Chassagne F, Gardes M (2013) Taxonomy of *Alnus*-Associated Hypogeous Species of *Alpova* and *Melanogaster* (*Basidiomycota*, *Paxillaceae*) in Europe. *Cryptogamie Mycologie* 32(1): 33-62. doi:10.7872/crym.v32.iss1.2012.033
16. Moreau PA, Welti S, Perić B, Jargeat P, Manzi S, Vizzini A (2012) *Alpova komoviana* (*Boletales*, *Paxillaceae*), a new sequestrate fungus from Montenegro, with a revised phylogeny of the genus in Europe. *Mycological Progress* 12:109-119. doi:10.1007/s11557-012-0818-x
17. Nilsson RH, Kristiansson E, Ryberg M, Larsson KH (2005) Approaching the taxonomic affiliation of unidentified sequences in public databases – an example from the mycorrhizal fungi. *BMC Bioinformatics* 6:178. doi:10.1186/1471-2105-6-178
18. Nylander JAA (2004) Mr Modeltest 2.2. Computer software distributed by the University of Uppsala. Uppsala, Sweden: Evolutionary Biology Centre, University of Uppsala.
19. Orihara T, Smith ME, Shimomura N, Iwase K, Maekawa N (2012) Diversity and systematics of the sequestrate genus *Octaviania* in Japan: two new subgenera and eleven new species. *Persoonia*, 28(6):85-112. doi:10.3767/003158512X650121
20. Page RD (2001) *TreeView*. Glasgow University, Glasgow.
21. Rambaut A (2000) Estimating the rate of molecular evolution: incorporating non-contemporaneous sequences into maximum likelihood phylogenies. *Bioinformatics* 16:395-399. doi:10.1093/bioinformatics/16.4.395
22. Ronquist F, Huelsenbeck JP (2003) MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574. doi:10.1093/bioinformatics/btg180
23. Ryberg M, Kristiansson E, Sjökvist E, Nilsson RH (2009) An outlook on the fungal internal transcribed spacer sequences in GenBank and the introduction of a web-based tool for the exploration of fungal diversity. *New Phytologist* 181:471–477. doi:10.1111/j.1469-8137.2008.02667.x
24. Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690. doi:10.1093/bioinformatics/btl446
25. Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. doi:10.1093/bioinformatics/btu033
26. Stamatakis A, Ludwig T, Meier H (2005) RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics* 21:456–463. doi:10.1093/bioinformatics/bti191
27. Teng SC (1963) *Fungi of China*. Beijing, China: Science Press, p. 660.
28. TÜRKOĞLU A, CASTELLANO MA (2013) New records of truffle fungi (*Basidiomycetes*) from Turkey. *Turkish Journal of Botany* 37: 970-976. doi:10.3906/bot-1212-54

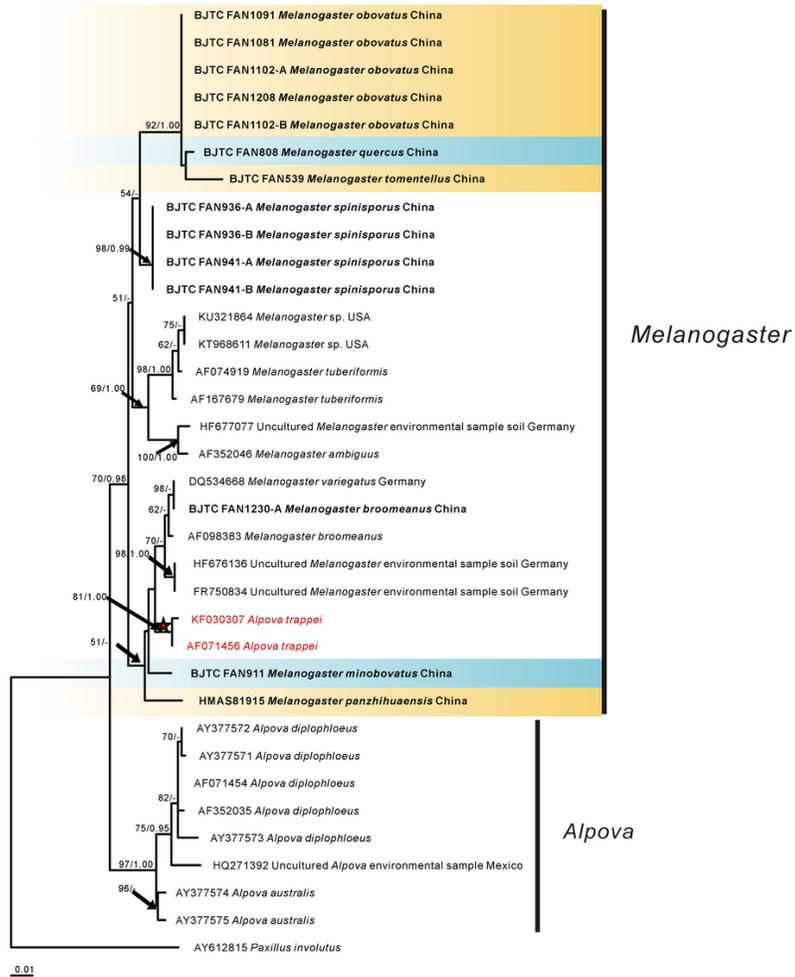


Figure 2

Phylogeny derived from maximum likelihood (ML) analysis of the nrLSU sequences from the *Melanogaster* and *Alpova* species. Values next to nodes reflect: left maximum likelihood bootstrap support values (BS) and right Bayesian posterior probabilities (PP). Names of novel species and samples with newly generated sequences in bold.

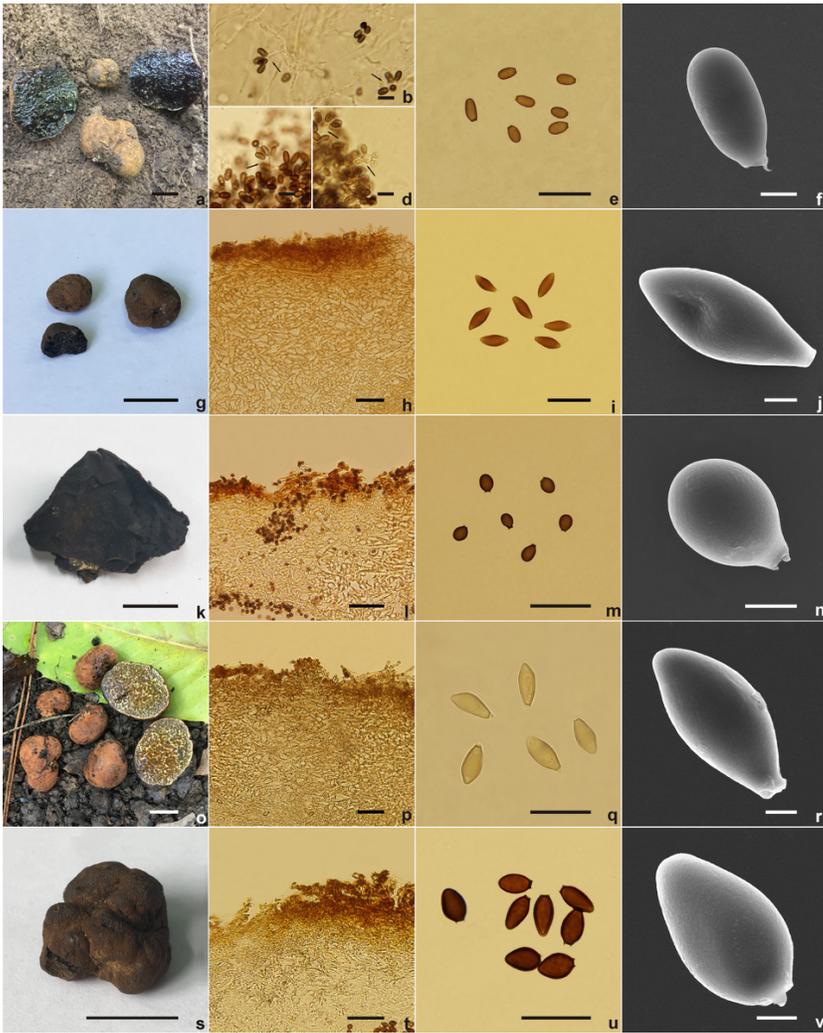


Figure 3
 Photographs of *Melanogaster* species. *Melanogaster broomeanus*. a Basidiomata (BJTC FAN1230). b-d. LM of basidia and basidiospores (BJTC FAN909). e. LM of basidiospores (BJTC FAN1230). f. SEM of ascospore (BJTC FAN1230). *Melanogaster fusisporus* (HMAS83274). g. Basidiomata. h. LM of Peridium. i. LM of basidiospores. j. SEM of ascospore. *Melanogaster minobovatus* (BJTC FAN911, holotype). k. Basidioma. l. LM of Peridium. m. LM of basidiospores. n. SEM of ascospore. *Melanogaster obovatus* (BJTC FAN1091, epitype). o. Basidiomata. p. LM of Peridium. q. LM of basidiospores. r. SEM of ascospore. *Melanogaster panzhuhuaensis* (HMAS81915, holotype). s. Basidioma. t. LM of Peridium. u. LM of basidiospores. v. SEM of ascospore. Scale bars: a, g, k, o, s=1cm; b, c, d=10 μ m; e, i, m, q, u=20 μ m; f, j, n, r, v=2 μ m; h, l, p, t=50 μ m.

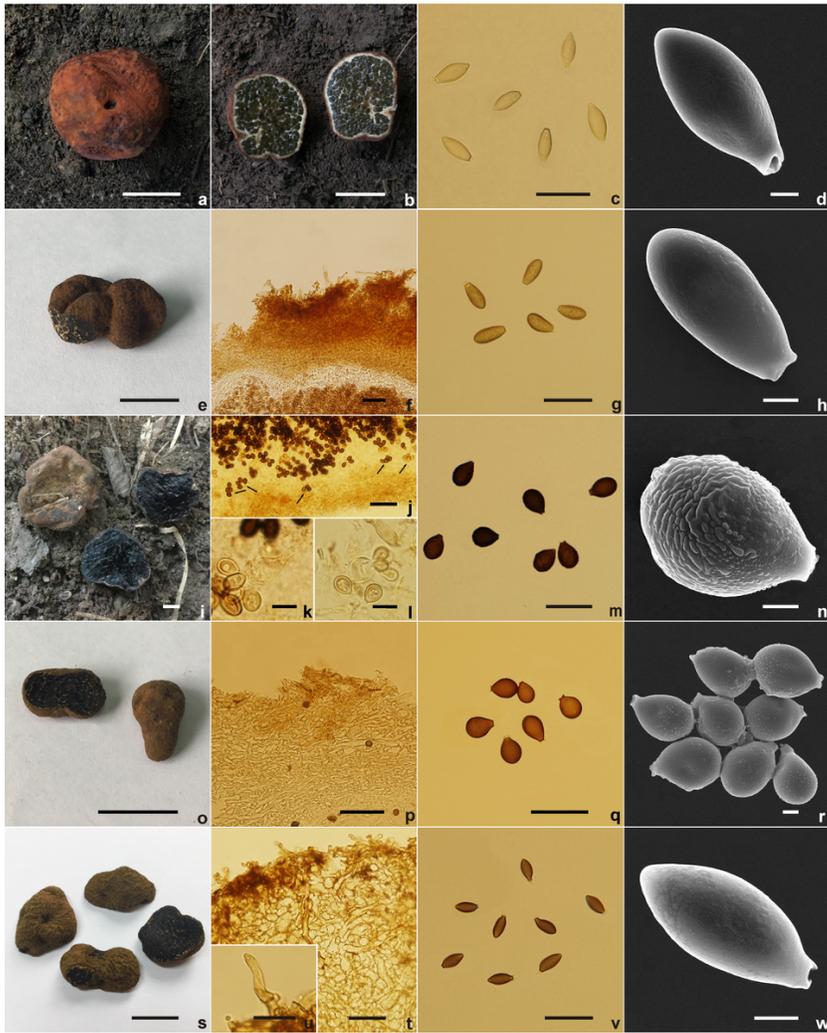


Figure 4

Photographs of *Melanogaster* species. *Melanogaster quercus* (BJTC FAN809, holotype). a-b. Basidiomata. c. LM of basidiospores. d. SEM of ascospore. *Melanogaster shanxiensis* (HMAS81910, holotype). e. Basidioma. f. LM of Peridium. g. LM of basidiospores. h. SEM of ascospore. *Melanogaster spinisporus* (BJTC FAN941-A, epitype). i. Basidiomata. j-l. LM of basidia and basidiospores. m. LM of basidiospores. n. SEM of ascospore. *Melanogaster subglobisporus* (HMAS81919, epitype). o. Basidiomata. p. LM of Peridium. q. LM of basidiospores. r. SEM of ascospores. *Melanogaster tomentellus* (BJTC FAN539, holotype). s. Basidiomata. t. LM of Peridium. u. LM of cystidia. v. LM of basidiospores. w. SEM of ascospores. Scale bars: a, b, e, i, o, s=1cm; c, g, m, q, v=20 μ m; d, h, n, w=2 μ m; f, j, p, t=50 μ m; k, l, u=50 μ m; r=5 μ m.